volunteer group, our data include challenge with 2 different sources of endotoxin. Although the response to each endotoxin source was not statistically different, there were decreased PMNs present in the CCRE versus Sigma groups, which, when both groups are combined, modestly increases the variability of this measure. Because of this increased variability and smaller sample size, we recognize that a larger sample size may have revealed a statistically significant correlation that we did not observe for the normal cohort. Second, the group with asthma had increased PMN responses relative to normal volunteers, but these differences were not statistically significant. Third, our study population was predominantly female, and it is possible that female sex may be important in asthma and response to pollutants. Finally, it is important to note that all of the patients with asthma studied in these protocols had mild intermittent disease and required no chronic controller medication. Nonetheless, within the asthma group (all of whom underwent challenge with the same source of endotoxin), we observed a significant correlation between endotoxin induced increases in airway inflammation and BMI.

To our knowledge, this is the first report examining a relationship between BMI and inflammatory response to inhaled endotoxin in asthma. Endotoxin has been linked to asthma severity in ambient air and domestic and occupational environments and is a good model for acute events associated with asthma exacerbation. There are a number of potential mechanisms by which BMI may be linked to the inflammatory response to inhaled endotoxin in asthma. We have reported that allergic airway inflammation may modify mCD14 expression in airway macrophages and monocytes, which may enhance their response to endotoxin. It is important to note, however, that without allergic and nonatopic controls with asthma, it is presently unclear whether atopy, asthma, or the combination of the 2 is responsible for the differences we observed between our study cohorts. Our group has also observed that even with modest changes in BMI, there is increased particle deposition in the airways. Thus, in patients with asthma, increased deposition of endotoxin related to body mass may allow an increased effective airway dose of endotoxin to interact with primed monocytes and macrophages. It is also possible that increased BMI reflects changes in inflammatory biology reported to occur in obesity, including increased leptin, which has recently been reported to promote eosinophil survival. Thus, obese patients with asthma may have modified response to endotoxin at a cellular level.

Regardless of the precise mechanism, it is important to consider the potential interaction between body mass and asthma exacerbation. Obesity likely represents an important target for intervention in many patients with moderate and severe asthma. Our data demonstrate that in asthma, further examination of both the effect of BMI on airway physiology relative to deposition of inhaled bioactive particles, as well as the effect of obesity on innate and acquired immune responses, is needed to understand the role of obesity in asthma pathogenesis and exacerbation.

*Neil E. Alexis, PhD*
*David B. Peden, MD*

From the Center for Environmental Medicine, Asthma and Lung Biology, Division of Pediatric Immunology and Infectious Diseases, Department of Pediatrics, 104 Mason Farm Road, CB#7310, University of North Carolina School of Medicine, Chapel Hill, NC 27516-7310.

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## Dominance of human innate immune responses in primary *Francisella tularensis* live vaccine strain vaccination

*To the Editor:*

*Francisella tularensis* is the etiologic agent of the zoonotic disease, tularemia. An inoculum as small as 10 bacteria can cause a flulike disease with substantial morbidity and mortality among infected humans.[1,2] Human tularemia presents in ulceroglandular, oculoglandular, oropharyngeal, pneumonic, and septic forms.[1,3] Rapid administration of antibiotics prevents mortality in the majority of human cases if exposure doses are low and nonaerosol.[2-4] Without early diagnosis and administration of antibiotics, high-dose aerosol exposure progresses rapidly to life-threatening pleuropneumonitis and systemic infection.[3] The relative abundance of *F tularensis* in nature and the relative ease with which it may be administered raise concerns over its exploitation as a biothreat agent.[1,3,4]

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In the 1950s, an attenuated strain of *F tularensis* was developed into an Investigational New Drug status live vaccine (live vaccine strain [LVS]) administered by intradermal scarification.[1,5,6] LVS vaccination has significantly lowered reports of laboratory-acquired tularemia, although the mechanism of protection (humoral or cell-mediated) is still unclear.[1,3,7] Microagglutination assays performed at 28 days postvaccination (indicating anti–*F tularensis* IgG and IgM) are the clinical standard for gauging successful vaccination yet show poor correlation with specific, vigorous lymphocyte responses in LVS-vaccinated and naturally infected humans.[8] Human anti–*F tularensis* immune serum resulting from vaccination with LVS is protective only against strains of reduced virulence, yet immunospecific and long-lasting cell-mediated immunity is the key to protection.[5,7,9] Substantial data suggest that cell-mediated immunity may be more important than humoral immunity for providing long-lasting immunity against virulent strains of *F tularensis*.[5,7,9] Therefore, we examined human immune responses to LVS vaccination to establish early cellular correlates of LVS-mediated protection predictive of successful vaccine outcomes.

Volunteers were recruited from US Army Medical Research Institute of Infectious Diseases (USAMRIID) personnel at risk of laboratory exposure to *F tularensis*. A minimal risk protocol to collect peripheral blood samples was approved by institutional review boards at the USAMRIID (Human Use Committee FY04-16). Donors provided informed consent and met eligibility criteria. Six healthy adults (4 males and 2 females, 22-54 years old) received a primary LVS vaccination and donated peripheral blood prevaccination and postvaccination. Mononuclear cells were purified by Ficoll gradients and assessed for changes in immune cell populations using flow cytometry and quadruple stained using directly conjugated mAbs (BD-Immunocytometry Systems and BD-Pharmingen, La Jolla, Calif). Cytometric bead array analysis was performed on serum samples (BD-Pharmingen). Analysis was performed using FlowJo (TreeStar, Inc, Ashland, Ore) and GraphPad Prism (GraphPad Prism Software, San Diego, Calif). All 6 subjects were immunologically naive before vaccination. All vaccinations had positive responses as indicated by initial formation of a small pustule/papule and subsequent ulceration.

Cell surface analysis of mononuclear cells revealed bias toward activation of innate versus acquired immune system components. The greatest changes in cellularity occurred on day +1 ($P < .001$ for day +1 versus all other days), a time frame consistent with innate immunity, but too short for unprimed acquired responses (Fig 1, *A and B*). When acquired immune system cells ($CD3^+$, $CD4^+$, $CD8^+$, T-cell receptor [TCR] $\alpha\beta^+$, $CD45RO^+$) were analyzed over all time points, only $CD4^+$ and $CD8^+$ cells were significantly changed at day +1 ($P < .05$ and $P < .01$, respectively).

Although $CD4^+$ and $CD8^+$ cells increased significantly on day +1 (Fig 1, *A*), these cells were not conventional $TCR\alpha\beta^+$ T cells, but rather $TCR\gamma\delta^+$ T cells ($CD8^+$/$\gamma\delta TCR^+$), natural killer (NK) T cells ($CD56^+$/$CD8^+$), and monocytes ($CD4^+$/$CD14^+$) whose kinetics mirrored innate immune responses (Fig 1, *B*). When innate immune system components ($CD56^+$, $CD1a^+$, $TCR\gamma\delta^+$, human leukocyte antigen [HLA]-$DR^+$, $CD16^+$, $CD14^+$) were tracked over the course of the vaccination, NK cells, γδT cells, monocytes, granulocytes, and dendritic cells showed considerable changes in cellularity on day +1. Dramatic increases in γδT cells (Fig 2, *A*) and NK cells (Fig 2, *B*) were noted on day +1 ($P < .05$ and $P < .01$, respectively), with NK cells having the most prominent changes. LVS vaccination induced a strong proliferative signal for innate lymphocytes as measured by the upregulation of the IL-2 receptor high-affinity α chain, CD25 (Fig 2, *C*), whose induction is linked with progression into cell cycle.[10] CD25 upregulation was highest on day +1 for all donors ($P < .01$) and was noted for NKT, γδT, and NK cells. No similar correlations were seen between CD25 and $TCR\alpha\beta^+$-$CD4^+$ or $TCR\alpha\beta^+$-$CD8^+$ T cells, even at time points associated with primary acquired immune responses, days +8 to +14.

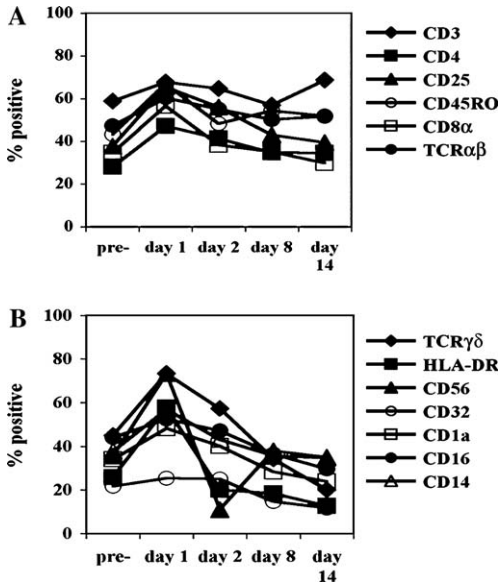


**FIG 1.** Cellularity changes in acquired and innate immune response to LVS vaccination. Peripheral blood was monitored for changes in acquired **(A)** and innate **(B)** immune cell surface markers over the course of several days prevaccination and postvaccination (as indicated). *Symbols* represent the mean percentage positive cells with n = 6.

Our data strongly suggest that cellularity changes after human LVS vaccination strongly parallel innate immune system kinetics. These data are in accord with the murine LVS model in which mice deficient in T cells are still able to resist lethal LVS challenge for 3 to 4 weeks.[5] This protection can be traced to proinflammatory cytokine production by innate immune components, chiefly NK and NKT cells.[5] Although serum cytokine levels in our study were below the limit of detection (<20 pg/mL), the upregulation of the high-affinity IL-2 receptor chain (CD25) may indicate that a proinflammatory $T_H1$-type response was

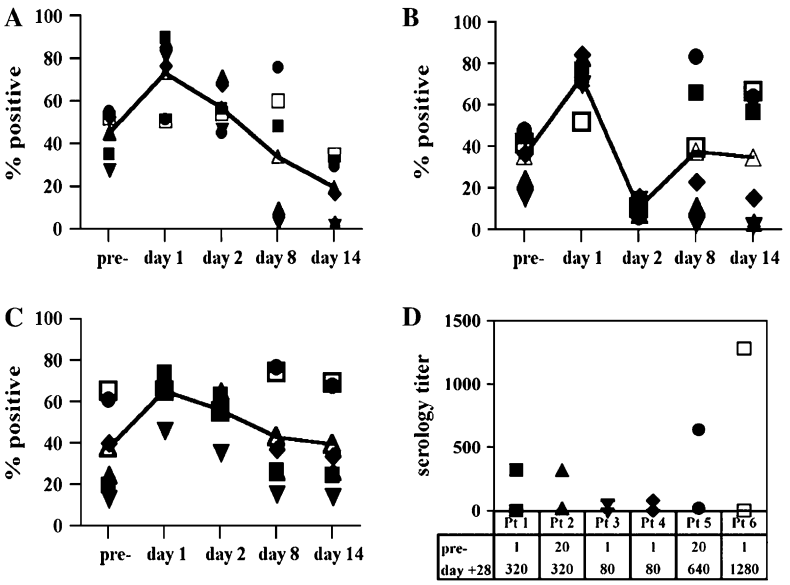


**FIG 2.** LVS vaccination promotes strong innate immune responses. PBMCs were analyzed for cell surface markers indicating γδT cells **(A)**, NK cells **(B)**, and competence for proliferation, CD25 **(C)**. Data represent individual patient values for each marker with a *line* connecting the average value for all 6 patients over time. **D,** Serologic titers for individual patients, prevaccination and postvaccination. *Symbols* represent individual patient titers and are consistent with individual cellular responses indicated *(A-C)*.

induced by LVS vaccination. All 6 of the subjects had positive anti-LVS titers by +28 days, yet there was as much as a 16-fold difference in positive microagglutination titers (Fig 2, *D*). Cellular responses at day +1 and +2 showed a much tighter cluster across all 6 subjects (less than 2-fold change between subjects), consistent with data suggesting the critical importance of cell-mediated immunity in long-term anti–*F tularensis* protection.[5,7,9] Surprisingly, postvaccination titer bore no resemblance to the cellular immune response described (Fig 2, *D*). Combined with data suggesting cell-mediated responses are most critical to anti–*F tularensis* protective immunity, the question of the role of humoral immunity remains unanswered.[5,7,9] Most importantly, our data point to cellular correlates of protection predictive of positive vaccine outcomes as early as 24 hours postinfection. Future studies to determine the gene-level responses to human LVS vaccination are underway in our laboratory.

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*Claudette L. Fuller, PhD**
*Katherine C. Brittingham, PhD**
*Matthew J. Hepburn, MD*
*James W. Martin, MD*
*Patricia L. Petitt, DO, MPH*
*Phillip R. Pittman, MD, MPH*
*Sina Bavari, PhD*

*These authors contributed equally to this work.

From the United States Army Medical Research Institute of Infectious Diseases, Bacteriology Division, 425 Porter St, Frederick, MD 21702-5011.
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